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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. LXI. OCCURRENCE OF CYCLOARTANE 6-O-MONODESMOSIDES

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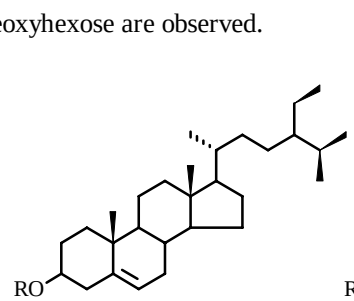
Cyclosiversigenin 6-O-α-L-rhamnopyranoside and 6-O-β-D-glucopyranoside were isolated from Astragalus coluteocarpus *Boiss. (Leguminosae) and* Astragalus dissectus *B. Fedtsch. et N. Ivanova, respectively. Cyclosiversigenin 5-O-α-L-rhamnopyranoside was shown to be an artifact for* Astragalus coluteocarpus. *Thus, the cyclosiversigenin 6-O-β-D-glucopyranoside that was isolated from certain* Astragalus *species is hypothesized also to be an artifact. Glycosylation of the 6α-hydroxyl group of cycloartanes by D-glucose and D-xylose, in contrast with other substituents, does not change the low-field position of the PMR signal of the 4α-CH_3 group ($1.65 \le \delta \le 2.01$ ppm) that is caused by the influence of deuteropyridine directly on the 6α-hydroxyl. Obviously one of the hydroxyls of the β-D-glucopyranoside or β-D-xylopyranoside residues has the same effect in this instance.*

Key words: *Astragalus*, cycloartanes, cyclosiversigenin, cyclosiversigenin 6-O-α-L-rhamnopyranoside, cyclosiversigenin 6-O-β-D-glucopyranoside.

The present article continues our study of triterpenes from species of *Astragalus* (Leguminosae) [1]. We previously isolated from *Astragalus coluteocarpus* Boiss. six compounds: cyclosiversigenin (**3**), β-sitosterol β-D-glucopyranoside (**2**), the cyclocarposides A (**9**), B (**10**), and C (**11**), and cyclocarposide (**12**) [2, 3]. We isolated from another batch of this same plant [4] the aforementioned compounds and another glycosidic compound **5**. GLC demonstrated that this glycoside contains one L-rhamnose residue [5]. This was concluded from the ^{1}H and ^{13}C NMR spectra of **5** (Table 1), in which signals from one 6-deoxyhexose are observed.



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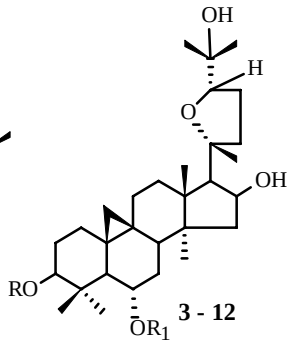

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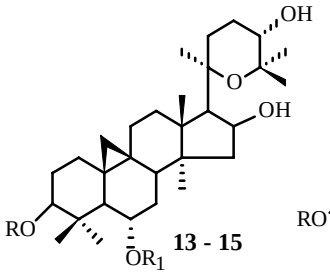
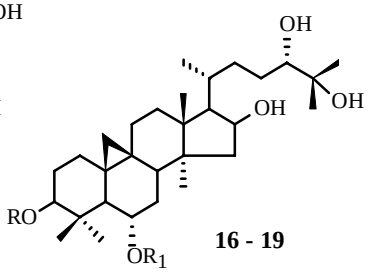


13 - 15



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1. R = H
2. R = β-*D*-Glc*p*
3. R = R_1 = H
4. R =β-*D*-Xyl*p*, R_1 = H
5. R = H, R_1 =α-*L*-Rha*p*
6. R = H, R_1 =β-*D*-Glc*p*
7. R = R_1 =β-*D*-Xyl*p*
8. R =β-*D*-Xyl*p*, R_1 =β-*D*-Glc*p*
9. R=β-*D*-(2-OAc)-Xyl*p*, R_1 =α-*L*-(2-OAc)-Rha*p*
10. R =β-*D*-Xyl*p*, R_1 =α-*L*-(2-OAc)-Rha*p*
11. R=β-*D*-(2-OAc)-Xyl*p*, R_1 =α-*L*-Rha*p*
12. R =β-*D*-Xyl*p*, R_1 = α-*L*-Rha*p*
13. R =R_1 =β-*D*-Xyl*p*
14. R =β-*D*-Xyl*p*, R_1 =β-*D*-Glc*p*
15. R =β-*D*-Glc*p*(1→2)-β-*D*-Xyl*p*, R_1 =β-*D*-Glc*p*
16. R =β-*D*-(4-OAc)-Xylp, R_1 =β-*D*-Glc*p*
17. R =β-*D*-Xyl*p*, R_1 =β-*D*-(6-OAc)-Glc*p*
18. R =β-*D*-Xyl*p*, R_1 =β-*D*-Glc*p*
19. R =β-*D*-Glc*p*(1→2)-β-*D*-Xyl*p*, R_1 =β-*D*-Glc*p*

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TABLE 1. Chemical Shifts of C Atoms in **3**, **5**, and **6** (C_5D_5N, 0 = TMS)

C atom	Compound		
	3	**5**	**6**
1	32.81	32.53	32.60
2	31.47	31.28	31.31
3	78.32	77.60	78.10
4	42.46	41.99	42.43
5	54.00	52.14	52.61
6	68.38	79.69	79.85
7	38.85	35.05	34.98[a]
8	47.30	46.86	46.30[b]
9	20.99	20.67	21.12
10	29.92	28.81	29.64[c]
11	26.32	26.43	26.48
12	33.47	33.35	33.46
13	45.09	45.04	45.11
14	46.21	46.24	46.30[b]
15	46.81	46.71	46.47
16	73.48	73.39	73.42
17	58.44	58.34	58.33
18	21.66	21.69	21.34
19	31.02	30.82	29.64[c]
20	87.27	87.19	87.27
21	28.59	28.56	28.59
22	34.97	34.93	34.98[a]
23	26.17	26.08	26.29
24	81.75	81.73	81.75
25	71.27	71.24	71.27
26	27.17	27.10	27.11
27	28.21	28.17	28.21
28	20.27	20.29	19.98
29	29.44	29.35	29.14
30	16.14	16.58	16.15
		α-*L*-Rha*p*	β-*D*-Glc*p*
1′		104.12	105.22
2′		72.97	75.60
3′		72.50	79.23
4′		73.76	71.92
5′		70.10	78.31
6′		18.19	63.15

*Signals marked with identical letters are superimposed.

The spectra suggest that the genin of the examined glycoside is cyclosiversigenin (**3**). The fact that the C-5 and C-7 signals in the ^{13}C NMR spectrum of **5** underwent high-field shifts compared with the signals of the analogous atoms in cyclosiversigenin and are observed at 52.14 and 35.05 ppm, respectively, is interesting. This unambiguously indicates that the L-rhamnose is bonded to the genin through the hydroxyl on C-6 [6]. Therefore, the signal of the latter undergoes a weak-field shift and appears at 79.69 ppm. It should be mentioned that the same atom of cyclosiversigenin resonates at 68.38 ppm. The good correspondence of the chemical shifts of the L-rhamnose C atoms in the ^{13}C NMR of **5** and those of cyclocarposide [2-4] indicates that the L-rhamnose in this glycoside is the pyranose isomer with $^1C_4$ conformation and α-configuration. Thus, the studied glycoside is cyclosiversigenin 6-O-α-L-rhamnopyranoside. We obtained a glycoside of identical structure as the progenin from cyclocarposide [4].

It should be noted that this monoside was not observed in the second batch of material [2]. Subsequent attempts to

observe **5** in other samples collected from the same sites and at the same vegetative stage were also unsuccessful. This tends to make us think that cyclosiversigenin 6-O-α-L-rhamnopyranoside is in all probability an artifact. This product could be formed during drying or storage of the material or the isolation. In the last instance, preparation of the aqueous extract creates conditions that are favorable for microbiological decomposition of the glycosides.

We isolated and identified β-sitosterol (**1**), cyclosiversigenin (**3**), β-sitosterol β-D-glucopyranoside (**2**), cyclosiversigenin 3-O-β-D-xylopyranoside (**4**), cyclosiversioside E (**7**), cyclodissectoside (**13**), cyclosiversioside F (**8**), and cyclocanthoside E (**18**) from *Astragalus dissectus* B. Fedtsch. et N. Ivanova [1, 7]. We isolated glycoside **6** from the fractions eluted after isolation of cyclosiversigenin 3-O-β-D-xylopyranoside. The ^{1}H and ^{13}C NMR spectra showed that this glycoside is also a cyclosiversigenin derivative that incorporates only β-D-glucopyranose. Atoms C-5, C-6, and C-7 appear at 52.61, 79.85, and 34.98 ppm, respectively, in the ^{13}C NMR of **6**. This is consistent with glycosylation of C-6 [6]. Thus, **6** is cyclosiversigenin 6-O-β-D-glucopyranoside, which has often been described as a progenin [8-10]. Cyclosiversigenin 6-O-β-D-glucopyranoside has subsequently been isolated from several *Astragalus* species [11-13].

Although it was not possible to re-examine various samples of this plant, we suppose from the aforementioned phenomena that this glycoside also is an artifact.

The signal for the 4α-CH_3 group in the PMR spectra of cycloartanes with a 6α-hydroxyl group that are recorded in deuteropyridine is known to be shifted to weak field and occurs in the range 1.65-2.0 ppm [14]. The physical interpretation of this phenomenon suggests that the deuteropyridine solvates the 6α-hydroxyl group through a H-bond and orients such that the 4α-CH_3 group becomes deshielded by the magnetically anisotropic aromatic ring of the deuteropyridine. Naturally any change that prevents the interaction of the deuteropyridine with the 6α-hydroxyl should eliminate this effect. In fact, alkylation, acylation, and oxidation to a ketone of the hydroxyl under discussion eliminates the weak-field shift of the signal for the 4α-CH_3 group [15, 16].

Glycosylation of the 6α-hydroxyl should similarly affect the chemical shift of the 4α-CH_3 group. However, the PMR spectra of most glycosides indicate that glycosylation does not unambiguously produce this effect but depends on the nature of the monosaccharide used. Thus, whereas a weak-field shift of the signal for the 4α-CH_3 group is not observed in the PMR spectra of glycosides containing L-rhamose on C-6 (**5**, **9**-**12**) [2-4], the signal lies in the range 1.86-2.01 ppm in the spectra of the 6-O-β-D-xylopyranosides **7** and **13** [1] and the 6-O-β-D-glucopyranosides **6**, **8**, and **14**-**19** [6, 8, 9, 17, 18]. It is noteworthy that the weak-field signal in the spectra of **14** and **15** was assigned to the 4α-CH_3 group based on HMQC and HMBC data [6, 17]. Obviously one of the hydroxyls in the carbohydrate framework of the 6-O-β-D-glucopyranosides and 6-O-β-D-xylopyranosides acts analogously to the 6α-hydroxyl of cycloartanes that orients the deuteropyridine.

EXPERIMENTAL

General comments have been reported [19]. We used the following solvent systems: 1) $CHCl_3$—CH_3OH (15:1) and 2) $CHCl_3$—CH_3OH—H_2O (70:12:1).

^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 and Bruker AC-200 spectrometers in deuteropyridine with TMS internal standard. ^{13}C NMR spectra were obtained with full saturation of C–H coupling and J-modulation.

Isolation of *Astragalus coluteocarpus* Isoprenoids. Slightly polar fractions that were obtained during isolation of cyclocarposide (**12**) [4] were rechromatographed on a column. The column was eluted successively with $CHCl_3$ and systems 1 and 2. Rechromatography of the collected fractions was performed by the literature method [2]. Compounds **2**, **3**, **9**-**12**, which were isolated previously [2], and glycoside **5** were isolated. The last is isolated from fractions containing **2** and after elution of **2**. This glycoside could not be found in other samples of the raw material.

Cyclosiversigenin 6-O-α-L-Rhamnopyranoside (5), $C_{36}H_{60}O_9$. The melting point and specific rotation have been reported [4]. GLC [5] showed that **5** contains one molecule of L-rhamnose.

PMR spectrum (200 MHz, C_5D_5N, 0 = TMS, δ, ppm, J, Hz): 0.28 and 0.46 (2H-19, d, ^{2}J = 4 Hz), 0.97, 1.15, 1.28, 1.30, 1.40, 1.44, 1.56 (7×CH_3, s), 1.59 (CH_3 of L-rhamnose, d), 2.52 (H-17, d, ^{3}J = 7.2 Hz), 3.10 (H-22, m), 5.0 (H-16, m), 5.37 (H-1′, br. s). For the ^{13}C NMR spectrum, see Table 1.

Isolation of *Astragalus dissectus* isoprenoids has been reported [7]. Glycoside **6** (27 mg) was obtained from the fraction eluted after cyclosiversigenin 3-O-β-D-xylopyranoside (**4**).

Cyclosiversigenin 6-O-β-D-Glucopyranoside (6), $C_{36}H_{60}O_{10}$. The melting point and specific rotation have been

reported [8-10].

PMR spectrum (400 MHz, C_5D_5N, 0 = TMS, δ, ppm, J, Hz): 0.27 and 0.63 (2H-19, d, ^2J = 4 Hz), 0.93, 1.28, 1.29, 1.39, 1.43, 1.56, 1.94 (7×CH_3, s), 2.51 (H-17, d, ^3J = 8 Hz), 3.11 (H-22, q, 1:3:3:1, $^2J = {}^3J_1 = {}^3J_2$ = 10 Hz), 3.58 (H-3, dd, $^3J_1$ = 12, $^3J_2$ = 5 Hz), 3.70 (H-6, m), 3.87 (H-24, H-5′, m), 4.02 (H-2′, t, $^3J_1 = {}^3J_2$ = 8 Hz), 4.18 (H-3′, H-4′, m), 4.30 (H-6′, dd, ^2J = 12, ^3J = 6 Hz), 4.46 (H-6″, dd, ^2J = 12 Hz, ^3J = 3 Hz), 4.92 (H-1′, d, ^3J = 8 Hz), 4.97 (H-16, q, 1:3:3:1, $^3J_1 = {}^3J_2 = {}^3J_3$ = 8 Hz). For the ^{13}C NMR spectrum, see Table 1.

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